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DATA REPORT

Novel compound heterozygous variants in the *LARP7* gene in a patient with Alazami syndrome

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The *LARP7* gene encodes a chaperone protein of the noncoding RNA 75 K, and mutations in this gene have been identified in patients with Alazami syndrome. Herein, we report another Japanese patient with Alazami syndrome and novel compound heterozygous variants in *LARP7* (i.e., c.370delG, p.Glu124fs*38 and c.641_667+25del involving the splice donor site of intron 8). These findings provide further evidence that biallelic LARP7 defects cause the phenotype of Alazami syndrome.

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Alazami syndrome is an autosomal recessive developmental disorder that is characterized by severe intellectual disability, postnatal growth retardation and distinct dysmorphic facial features that include a protruding forehead, deep-set eyes, narrow palpebral fissures, a broad nose and malar hypoplasia (OMIM #615071). This syndrome was first identified in a large consanguineous Saudi Arabian family in 2012. A homozygous frameshift mutation in the La ribonucleoprotein domain family member 7 gene (*LARP7*), which encodes a chaperone protein of the noncoding RNA 75 K, was identified in all of the affected patients in the family.[1]

Because only four families with Alazami syndrome and *LARP7* mutations have been reported thus far, the phenotypic spectrum of LARP7-related disease remains unclear.[1–4] Herein, we report an additional patient with Alazami syndrome with novel compound heterozygous mutations in the *LARP7* gene and review the clinical and genetic findings from patients with Alazami syndrome.

A male Japanese patient was born at 38 weeks of gestation after an uncomplicated pregnancy and delivery. At birth, his length was 46 cm (−1.3 standard deviation [SD]), and his weight was 2.44 kg (−1.1 SD). He was found to have subcoronal hypospadias and an inguinal hernia. He also exhibited distinct facial features that included a prominent forehead, narrow palpebral fissures, deep-set eyes, hypertelorism, a broad nose and malar hypoplasia (Figure 1a). He had severe hypotonia in infancy. His motor development was obviously delayed; he held up his head at 9 months, rolled over at 10 months and walked at 2 years. At the last examination at 2 years of age, he measured 80.6 cm (−1.14 SD) and weighed 9.45 kg (−1.28 SD). He could not speak any meaningful words and exhibited intellectual disability (total IQ, 67).

His non-consanguineous parents were clinically normal. His father and mother were 180 cm (+0.6 SD) and 152 cm (−1.3 SD) in height, respectively. There were no family histories of neurodevelopmental diseases or congenital malformations.

This study was approved by the Institutional Review Board of the Nagasaki University Graduate School of Biomedical Sciences. Because clinical assessment alone was unable to identify a conclusive diagnosis, we sought to identify disease-causing mutations with a trio whole-exome sequencing (WES) strategy using a SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA) on a HiSeq 2500 platform (Illumina, San Diego, CA, USA). Written informed consent was obtained from the parents. DNA samples were obtained from peripheral blood

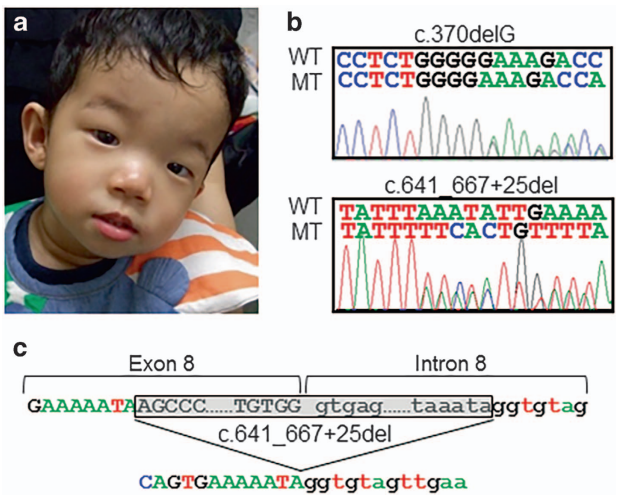

Figure 1. Clinical and genetic findings in the patient. (**a**) A front view of the patient at 2 years of age showing the distinct facial features of a prominent forehead, narrow palpebral fissures, deep-set eyes, hypertelorism, a broad nose and malar hypoplasia. (**b**) Electropherograms displaying the two *LARP7* mutations in the patient. The upper (c.370delG) and lower (c.641_667+25del) sequences are the sense and antisense sequences, respectively. WT, wild-type allele; MT, mutant allele. (**c**) A schematic representation of the 52-bp deletion (c.641_667+25del) in *LARP7*. The exonic and intronic sequences are indicated by capital and lower-case letters, respectively. The deleted sequences are shaded in gray. The deletion involved the splice donor site of intron 8 and was predicted to cause an exon skipping of exon 8.

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Table 1. Clinical and genetic features of patients with Alazami syndrome

		Alazami *et al.* [1]									Ling *et al.* [2]	Holink *et al.* [3]		Holohan *et al.* [4]	Present case	
Case #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Age at the last examination (yrs)		12	5	22	20	15	17	10	8	5	2.5	6	2.5	8	2	
Sex		Male	Female	Male	Male	Male	Female	Male	Female	Female	Female	Male	Male	Male	Male	
LARP7 mutations																
cDNA					c.1024_1030dup (homo)					c.213_214dup c.651_655del	c.1091_1094del (homo)	c.1045_1051dup (homo)	c.756_757del (homo)	c.370del G c.641_667+25del		
Protein					p.Thr344Lysfs* (homo)					p.Ser72fs p. Lys219fs	p.Arg364fs (homo)	p.Thr351fs* (homo)	p.Arg253fs* (homo)	p.Glu124fs p.Phe192fs		
Birth measurements																
Gestational age		ND	ND	ND	ND	ND	ND	ND	ND	ND	40 wks	40 wks 3 days	ND	ND	38 wks	
Weight (SD)		ND	ND	ND	ND	ND	ND	ND	ND	ND	2608 g (−1.35)	2980 g	2000 g	ND	2444 g (−1.1)	
Height (SD)		ND	ND	ND	ND	ND	ND	ND	ND	ND	47.6 m (−0.91)	45 cm	ND	ND	46.3 cm (−1.3)	
OFC (SD)		ND	ND	ND	ND	ND	ND	ND	ND	ND	32.4 cm (−1.35)	34 cm	ND	ND	33 cm (−0.1)	
Present measurements																
Weight (SD)		14.6 kg (−3.3)	8 kg (−4.0)	33 kg (−4.5)	37 kg (−4.0)	31 kg (−3.5)	24 kg (−3.9)	25 kg (−2.0)	13 kg (−3.4)	10 kg (−3.3)	7.6 kg (−5.5)	16.5 kg (−1.0)	9.1 kg (−3.0)	ND	9.75 kg (−1.1)	
Height (SD)		117 cm (−7.0)	90 cm (−4.0)	129 cm (−10.5)	138 cm (−9.7)	138 cm (−9.5)	133 cm (−5.6)	135 cm (−5.0)	109 cm (−3.6)	92 cm (−4.0)	72.7 cm (−4.0)	107.4 cm (−2.5)	81 cm (−3.0)	(< −3.5)	82 cm (−1.4)	
OFC (SD)		48 cm (−3.3)	40 cm (−7.0)	51 cm (−3.0)	53 cm (−2.0)	53 cm (−2.0)	49 cm (−3.3)	51 cm (−1.5)	49.5 cm (−1.5)	48 cm (−1.7)	46 cm (−1.0)	48.4 cm (−2.0)	44.5 cm (−4.0)	ND	45 cm (−1.5)	
Developmental status																
Motor developmental delay	100% (14/14)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intellectual disability	100% (14/14)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Clinical features																
Trianglar face	85% (11/13)		+	+	+	+	+	+	+	+	+	+	−	+	ND	−
Prominent forehead	100% (14/14)		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep-seated eyes	100% (13/13)		+	+	+	+	+	+	+	+	+	+	+	+	ND	+
Narrow palpebral fissures	69% (9/13)		+	+	+	+	+	−	+	−	+	−	−	+	ND	+
Sparse eyebrows	83% (10/12)		+	+	+	+	+	+	+	+	+	ND	−	+	ND	−
Broad nose	100% (14/14)		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Low-set ears	54% (7/13)		+	+	−	+	+	−	+	−	+	+	−	−	ND	−
Wide mouth	85% (11/13)		+	+	+	+	+	+	+	+	+	+	−	+	ND	−
Full lips	69% (9/13)		−	+	+	−	−	+	+	+	−	+	+	+	ND	+
Widely spaced teeth	77% (10/13)		+	+	+	+	+	−	+	+	−	+	+	+	ND	−
Malar hypoplasia	85% (11/13)		+	+	+	+	+	−	+	+	+	+	−	+	ND	+
Strabismus	33% (4/12)		−	+	+	+	−	−	+	−	−	ND	−	−	ND	−
Thickend skin over hands	33% (4/12)		+	−	+	−	−	−	+	−	−	ND	+	−	ND	−
Scoliosis	25% (3/12)		+	+	−	−	−	−	−	−	−	ND	+	−	ND	−
Other findings							Short Achilles' tendon	Short Achilles' tendon			Blue/grey sclera, easily anxious and agitated	Clinodactyly of the big toes	Cleft palate, torticollis	Hypertelorism, hypotonia, small hands	Hypospadias, inguinal hernia, hypertelorism hypotonia	

Abbreviations: ND, not described; OFC, occipitofrontal circumference; SD, standard deviation; +, present; −, absent.

samples from the patient and the parents. The reads in the FASTQ files were aligned to the human reference genome using Novoalign version 3.0 (http://www.novocraft.com/). The trio-based genomic variation information was detected with the Genome Analysis Toolkit software version 3.4–46.[5] Subsequently, *de novo*, homozygous, heterozygous and X-linked variations were extracted and annotated with the ANNOVAR software.[6] This process excluded variants with allele frequencies $>0.5\%$ in any of the following databases: the Exome Aggregation Consortium (http://exac.broadinstitute.org/), the NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp) and the 1KJPN database of the Tohoku Medical Megabank (http://www.dist.megabank.tohoku.ac.jp). Heterozygous variations sharing the same GENCODE v19 genes were also extracted to detect compound heterozygous mutations. The mutations were confirmed via Sanger sequencing using a BigDye terminator and a 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

Utilizing the aforementioned strategy, we identified several candidate variants. Of these variants, compound heterozygous mutations in *LARP7* (c.370delG and c.641_667+25del involving exon 8 and intron 8; NM_001267039) were proposed as the best candidates based on mutual references to the WES data and the Online Mendelian Inheritance in Man database information of known diseases (www.omim.org) (Figure 1b). The father and mother of the proband were heterozygous for the c.370delG and c.641_667+25del variants, respectively. These two variants have not been recorded in the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/) or the Exome Aggregation Consortium Database (http://exac.broadinstitute.org/). A 1-bp deletion in exon 6 (c.370delG) was predicted to cause a frameshift at codon 124 in *LARP7* and a consequent termination at codon 161 (p.Glu124fs*38; NP_001253968). The deletion involving the splice donor site of intron 8 was predicted to cause an exon skipping of exon 8 during transcript processing, a frameshift mutation at codon 192 of *LARP7* and a resultant termination at codon 204 (p.Phe192fs*13) (Figure 1c).

The *LARP7* mutations identified in the present patient as well as the previously reported mutations are all frameshift mutations that are thought to cause early truncation of the LARP7 protein and thereby induce nonsense-mediated mRNA decay (Table 1).[7] This supposition suggests that a severe loss-of-function of LARP7 is likely the main mechanism underlying the phenotype of Alazami syndrome.[1–4]

A comprehensive comparison of the clinical findings between the present patient and the previously reported cases revealed several important points (Table 1). First, severe intellectual disability, motor developmental delay and characteristic facial features, including deep-set eyes, a broad nose, narrow palpebral fissures, a prominent forehead and malar hypoplasia, are common characteristic findings in patients with biallelic *LARP7* mutations. Second, although normal prenatal growth has been observed in some afflicted patients, severe postnatal growth disturbances that range from -2.5 to -10 SD have been found to be common among the previously reported patients. However, the phenotypic variety may be broader than initially presumed. Indeed, the present patient exhibited a relatively normal height up to 2 years of age. Third, the present patient has hypospadias, which has not been observed in any previously reported patients. Hypospadias is a common congenital malformation of the male external genitalia that is characterized by an aberrant opening of the urethra on the ventral side of the penis. While several genes, such as *SRD5A2*, *AR*, *HOXA13* and *MAMLD1*, have been identified as causative genes in hypospadias, pathogenic mutations have thus far been identified in only a very small portion of patients with hypospadias.[8] This finding is consistent with the notion of hypospadias as a highly heterogeneous condition that involves multiple genetic and environmental factors. Although whether this phenotype is a part of the spectrum of the manifestations of *LARP7* mutations remains to be determined, the present study may broaden the genetic variability that is associated with then occurrence of hypospadias.

In conclusion, our study has provided further evidence that biallelic LARP7 defects cause Alazami syndrome. Further studies are needed to determine the clinical spectrum of patients with Alazami syndrome and the pathogenesis of *LARP7* mutations.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.1923, http://dx.doi.org/10.6084/m9.figshare.hgv.1926.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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